

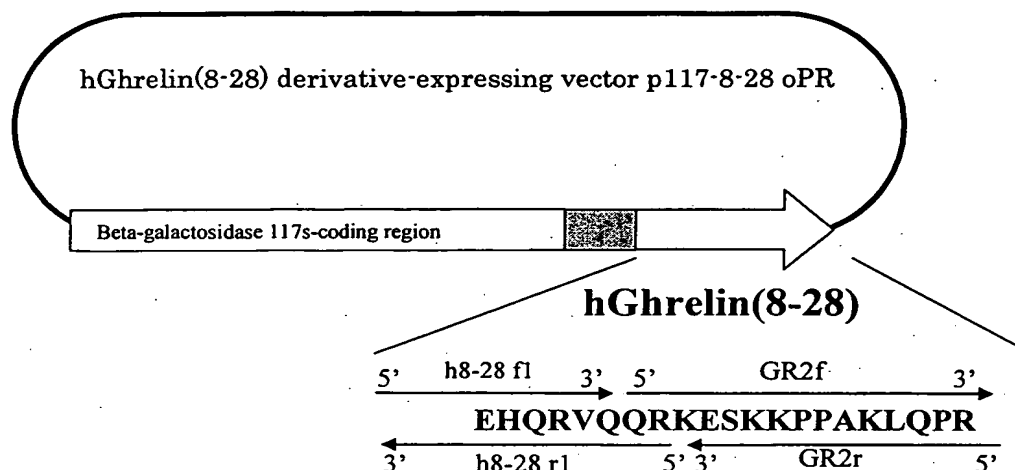


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Fig. 1

A



h8-28 fl 5'- TCCCCGCGGGAACACCAGCGCGTCCAG -3'

GR2f 5'- CAGCGTAAGGAATCCAAGAAGCCACCAGCTAAACTGCAGCCACGATAG -3'

GR2r 5'- TCGACTCATCGTGGCTGCAGTTTAGCTGGCTTCTTGGATTCCCT -3'

h8-28 r1 5'- ACGCTGCTGGACGCGCTGGTGTTCCCGCGGGA -3'

Synthetic oligo-DNAs used in annealing method

- h8-28 fl, GR2f, h8-28 r1 and GR2r are synthetic oligo-DNA nucleotide sequence.
- Bold underlined bases in GR2f and GR2r sequences are termination codon and cohesive end of SalI cleavage site.
- Bold underlined bases in h8-28 fl and h8-28 r1 sequences correspond to SalI cleavage site.

 corresponds to a linker sequence EPHHHHPGGRQMhGYDADVRLYRRHHGSGSPSRHRR.

B

| | | |
|-------------------------|-----------------|----------------|
| Beta-Galactosidase 117s | Linker sequence | hGhrelin(8-28) |
|-------------------------|-----------------|----------------|

Linker sequence EPHHHHPGGRQMhGYDADVRLYRRHHGSGSPSRHRR

hGhrelin(8-28) EHQRVQQRKESKKPPAKLQPR



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Fig. 2

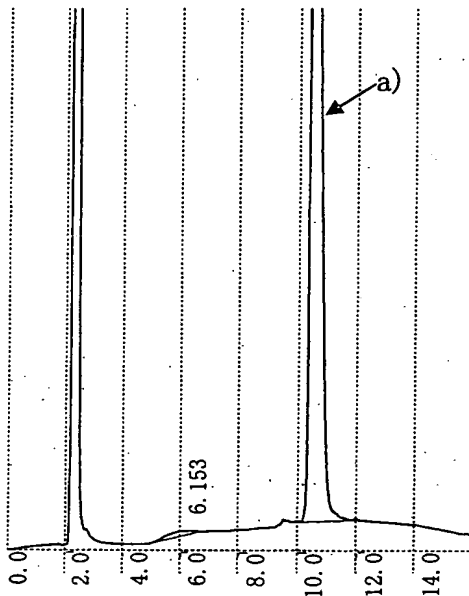


Fig. 3

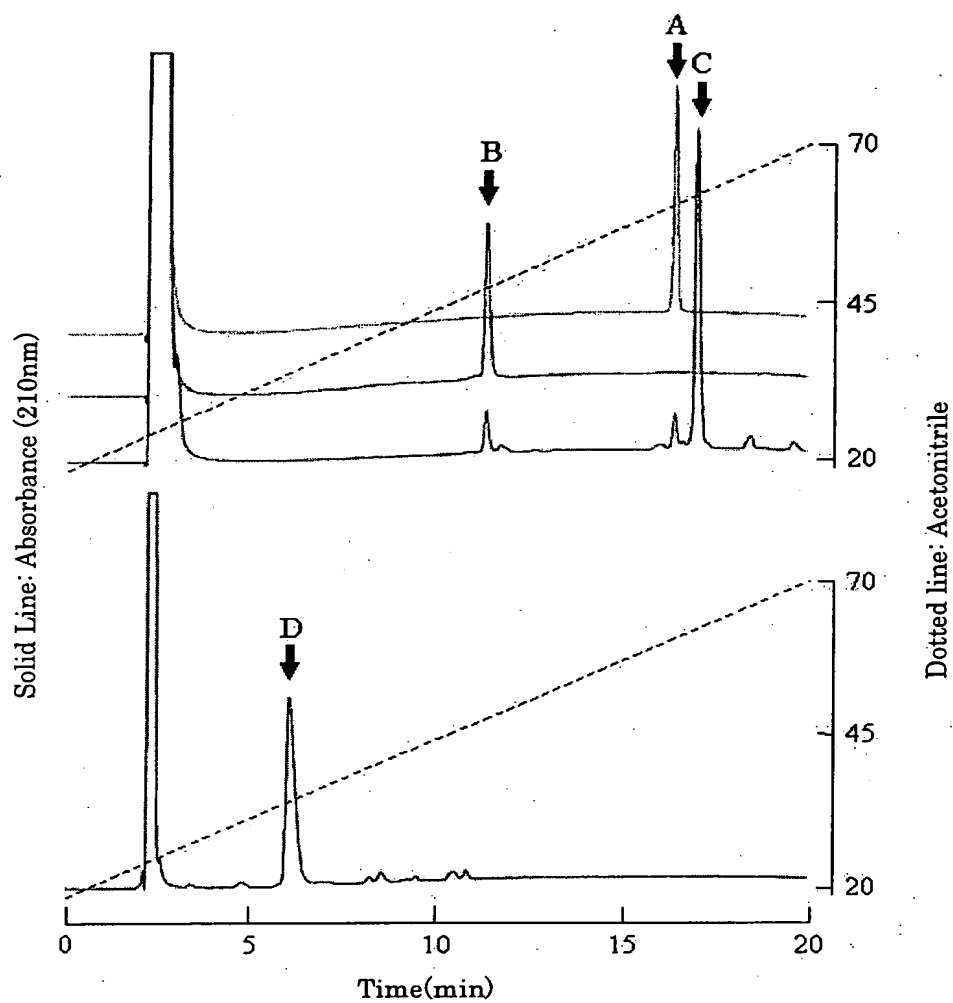




Fig. 4

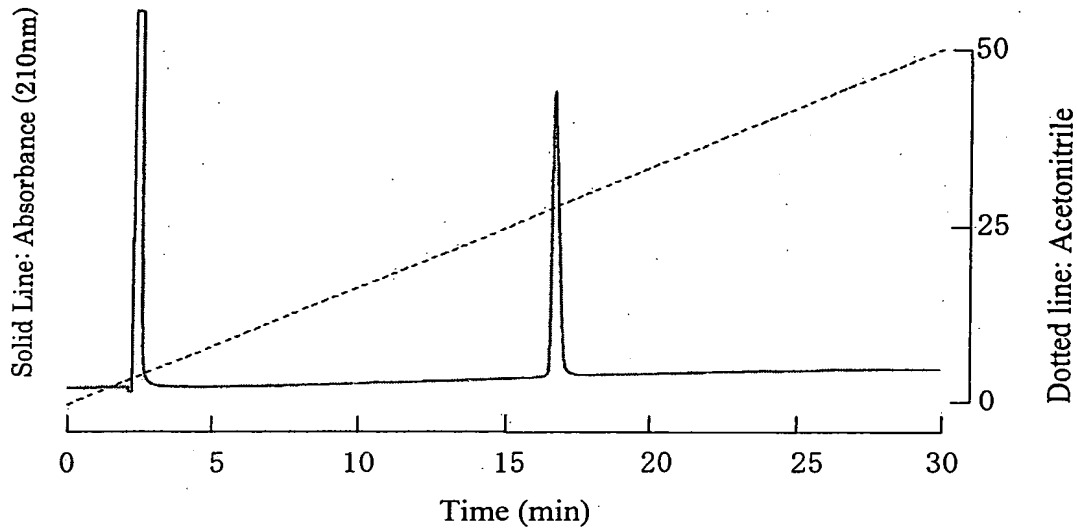
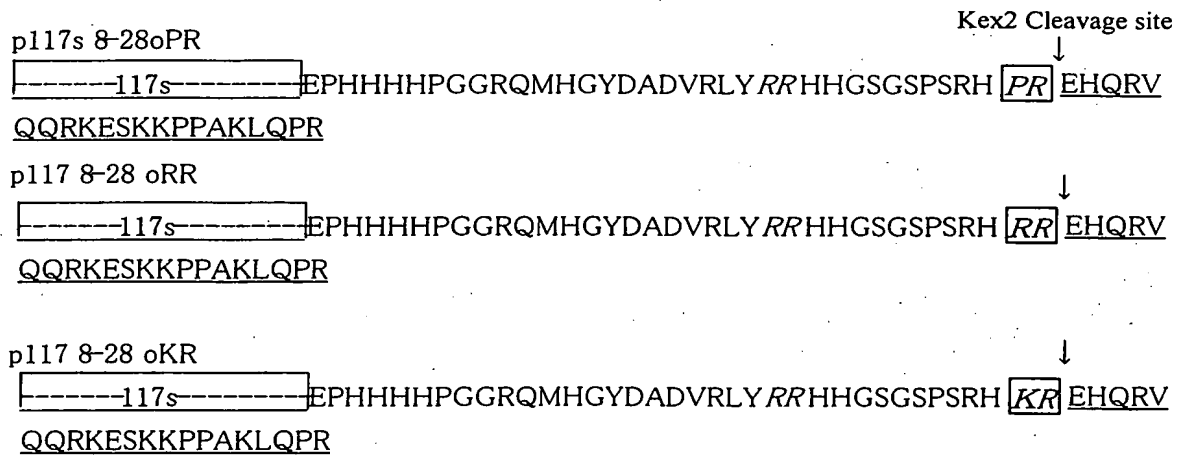


Fig. 5



-----117s----- is beta-galactosidase 117 amino acids

Underlined part is hGhrelin (8-28)

 represents a recognition site of Kex2, and ↓ represents a cleavage site of Kex2.



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Fig. 6

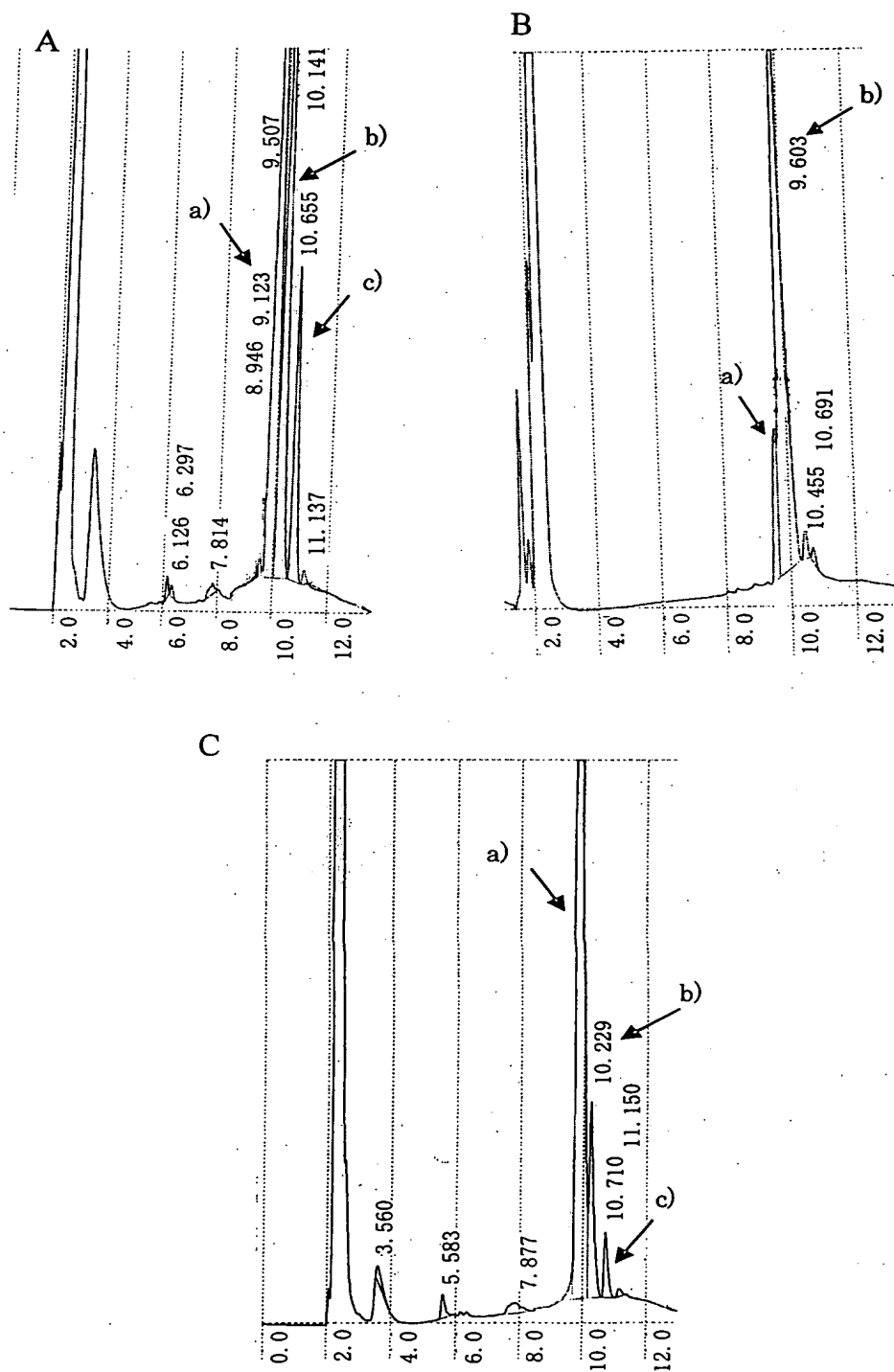


Fig. 7

117s 8-28 oRR

Fusion protein expressed in p117 8-28 oPR

-----117s-----EPHHHHHPGGRQMHGYDADVRLY RR HHGSGSPSRH RR EHQRV
QQRKESKKPPAKLQPR

117s PE

-----117s-----EPHHHHHPGGRQMHGYDADVRLY RR HHGSGSESRH RR EHQRV
QQRKESKKPPAKLQPR

117s SPEE

-----117s-----EPHHHHHPGGRQMHGYDADVRLY RR HHGSGEESRH RR EHQRV
QQRKESKKPPAKLQPR

117s SPEE+12aa

-----117s-----EPHHHHHPGGRQ MHGEDEDVVFVT MHGYDADVRLY RR

Kex2

HHGSGEESRH RR EHQRVQQRKESKKPPAKLQPR

117s VH

-----117s-----EPHHHHHPGGRQMHGYDADVRLY RR HHGSGSPSVH RR EHQRV
QQRKESKKPPAKLQPR

117s HV

-----117s-----EPHHHHHPGGRQMHGYDADVRLY RR HHGSGSPSHV RR EHQRV
QQRKESKKPPAKLQPR

117s FE

-----117s-----EPHHHHHPGGRQMHGYDADVRLY RR HHGSGSPSFE RR EHQRV
QQRKESKKPPAKLQPR

-----117s----- is beta-galactosidase 117 amino acids

Underlined part represents hGhrelin(8-28).

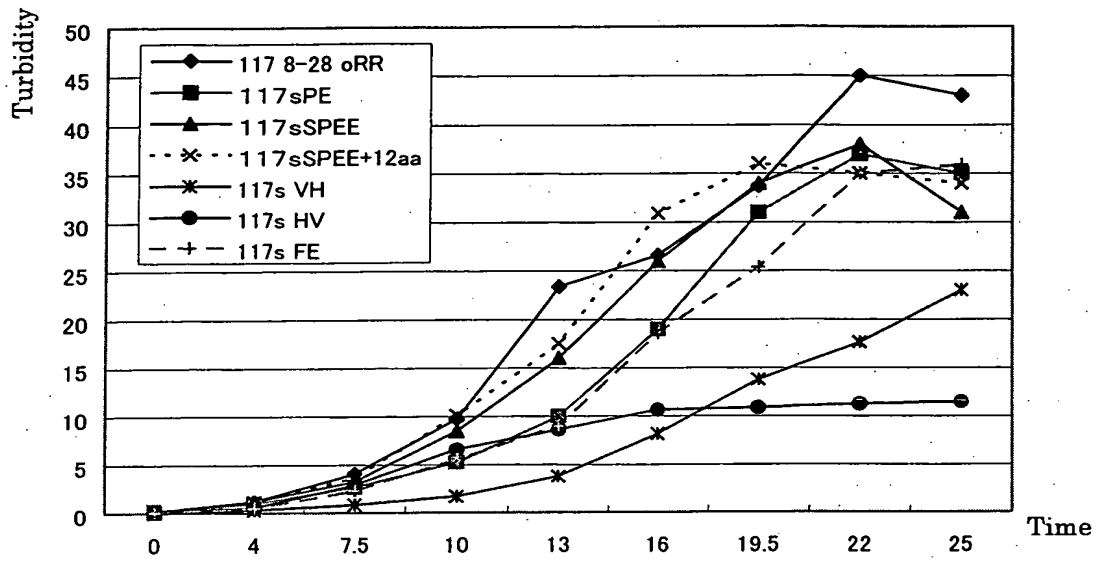
RR and RR represent cleavage recognition sites of OmpT and Kex2.

A slant underlined part represents a residue in which a mutation is introduced.



Fig. 8

A



B

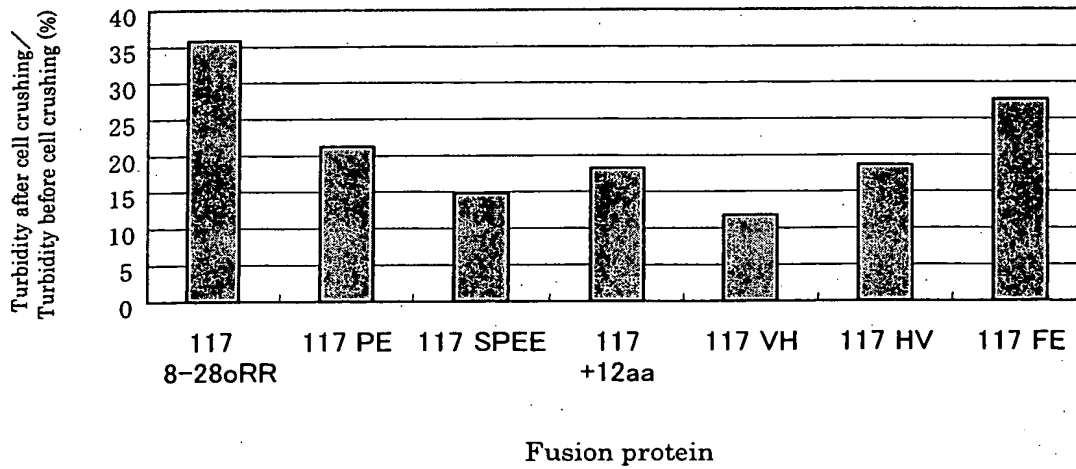


Fig. 9

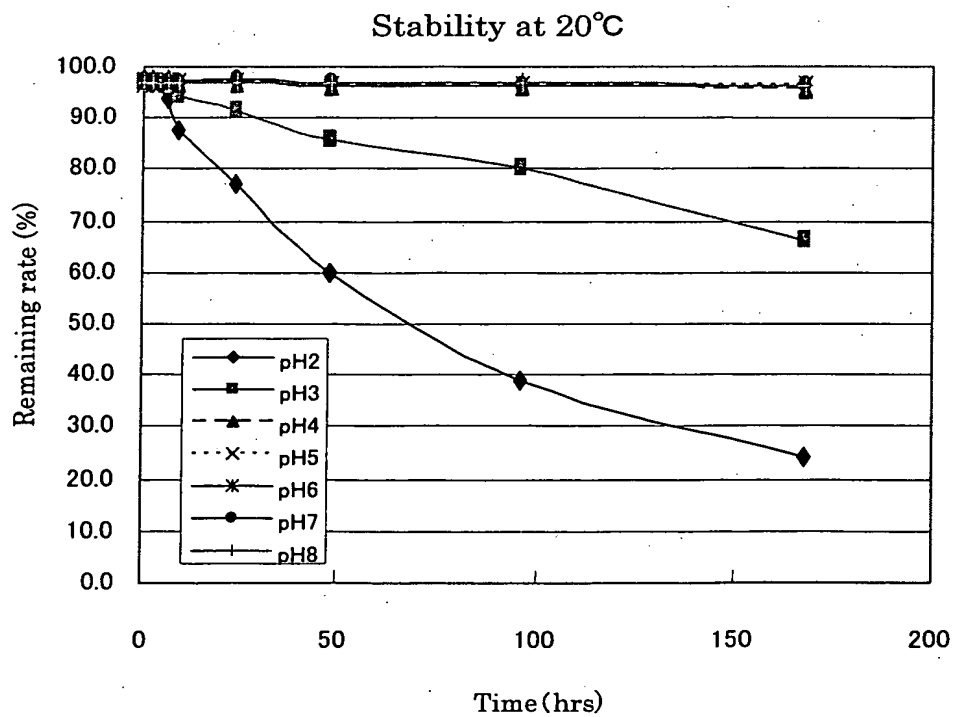
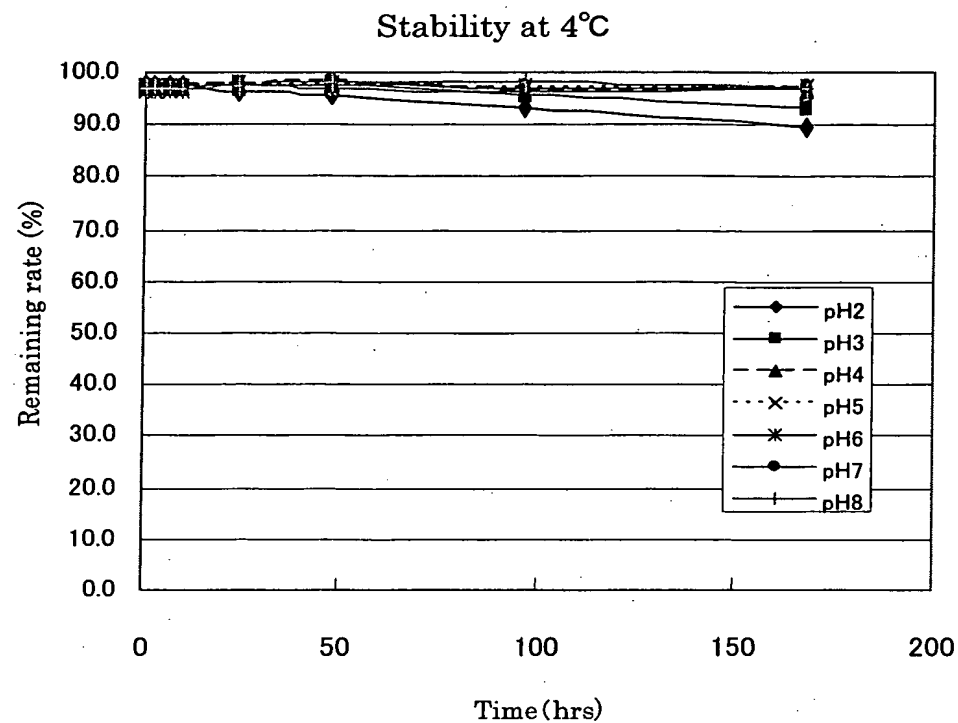




Fig. 10

